values is too large to be attributed solely to differences in sample temperatures and suggests at least some contribution to the relaxation arising from chemical-shift anisotropy. The chemical-shift anisotropy contribution cannot, however, be more than a few percent because the difference in T_1 values is 20% and $1/T_{1,CS}$ has a quadratic dependence on field strength.

The T_1 of $^{15}N_{\omega,\omega'}$ in arginine–glycerol solution was not determined at 50.65 MHz because the resonance broadens as N_ω and $N_{\omega'}$ become chemically nonequivalent as the result of hindered rotation around the N_δ=C bond. At 10 °C, this broadening occurs only when the spectrum is measured at 50.65 MHz, where the chemical-shift difference between N_ω and $N_{\omega'}$ (~90 Hz) is of the same order of magnitude as the reciprocal of the lifetime before rotation about the N_δ=C bond. In all T_1 experiments at 18.25 MHz, the $N_{\omega,\omega'}$ resonances were sharp with line widths of approximately 10 Hz (see Figure 2A).

In summary, the results indicate that, while the interpretation of the T_1 values of N_α and N_δ of arginine must take into account the contribution of nondipolar relaxation mechanisms, the T_1 values of $N_{\omega,\omega'}$ of arginine, N_γ of glutamine, and N_α of alanine, which are predominantly the result of dipolar relaxation, provide useful information about the microviscosities of their various environments and the association with other cellular components. Furthermore, the results suggest either that the vacuolar viscosity is substantially larger than that of the cytoplasm or that the ω,ω'-nitrogens of intracellular arginine are highly associated with polyanions (e.g., polyphosphates). The possible role of polyphosphates in binding vacuolar arginine can be examined by determining the effect of growth on limited phosphate on the T_1 value of $N_{\omega,\omega'}$ of intracellular arginine. If the T_1 is short in the absence of polyphosphate, this will suggest a highly viscous vacuolar environment or an association of arginine with other polyanions. This study is now under way.

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Studies of the Binding Interactions of *cis*-Diamminedichloroplatinum(II) with Amines and Nucleosides by Nitrogen-15 Nuclear Magnetic Resonance†

Michael Nee and John D. Roberts*

ABSTRACT: The ^{15}N chemical shifts and couplings have been measured for several platinum(II)–amine complexes. The ^{15}N chemical shift changes found on coordination of azine-type nitrogens to platinum appear to be related to those that occur on protonation of the same nitrogens. Both the ^{15}N chemical shifts and the one-bond ^{15}N–^{195}Pt coupling constants depend on the nature of the cis and trans ligands. *cis*-$[Pt(NH_3)_2Cl_2]$ ("*cis*-platinum") forms a complex with cytidine through N3. Guanosine becomes bound to platinum of *cis*-$[Pt(NH_3)_2Cl_2]$ at N7 and at least one other nitrogen. Adenosine appears to bind *cis*-$[Pt(NH_3)_2Cl_2]$ to at least some degree through N1, N3, N7, and the 6′ $-NH_2$.

There has been much interest in the chemistry and biochemistry of platinum complexes since the discovery of the antitumor activity of *cis*-$[Pt(NH_3)_2Cl_2)$, "*cis*-platinum", by Rosenberg and co-workers (Rosenberg et al., 1969). This substance has proven to be an especially effective agent for treatment of cancer of genitourinary origin (Cleare & Hydes, 1980). It appears to act by combining with specific nitrogens of the nucleotide bases of deoxyribonucleic acid, DNA (Roberts & Thomson, 1979).

Platination of the DNA purine and pyrimidine bases has been studied previously by ultraviolet (Mansy et al., 1973) and Raman (Chu et al., 1977, 1978) spectroscopies, ^1H (Chu et al., 1977, 1978; Kong & Theophanides, 1974a,b, 1975) and ^{13}C NMR[1] spectroscopies (Chu et al., 1977; Lim & Martin,

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[1] Abbreviations: NMR, nuclear magnetic resonance; an, aniline; py, pyridine; EDTA, ethylenediaminetetraacetic acid; Cyt, cytidine; Guo, guanosine.

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1976), and X-ray diffraction (Gellert & Bau, 1975; Lock et al., 1976a,b; Louie & Bau, 1977; Cramer & Dahlstrom, 1977; Melanson & Rochon, 1978). Each of these methods has shortcomings. Thus, ultraviolet and ^1H and ^{13}C NMR spectroscopies provide rather indirect evidence as to the platination sites of the bases. The Raman data are also hard to interpret in terms of specific binding sites, and X-ray diffraction, while providing definite proof of the location of platination sites, can only be used on crystalline platinum–nucleoside complexes. Because the base nitrogens are expected to be the preferred platination sites (Marzilli et al., 1980), ^{15}N NMR provides an especially direct method for determining the binding interactions of *cis*-$[Pt(NH_3)_2Cl_2]$ with nucleosides. An earlier ^{15}N NMR study of ^{15}N-labeled *cis*-$[Pt(NH_3)_2(H_2O)]^{2+}$ and its complex with ^{15}N-labeled 1-methylimidazole (Alei et al., 1979), suggested that information about the binding interactions could be determined not only by observation of the base nitrogen resonances but also by observation of those of the complexed ammonias as well.

Experimental Procedures

The ^{15}N NMR spectra were taken with a Bruker WH-180 FT NMR spectrometer operating at 18.25 MHz and a Bruker WM-500 FT NMR spectrometer operating at 50.70 MHz. The spectral conditions for the measurement of the ^{15}N-labeled ammonia complexes were 9000-Hz spectral width, 8K data points, and broad-band proton noise decoupling. The spectral conditions for measurements of the nucleoside–platinum complexes at the natural-abundance level of nitrogen-15 were 9000-Hz (WM-500, 30 000-Hz) spectral width, 8K (WM-500, 16K) data points, 30° pulse angle, 20-s delay time, and gated decoupling (during acquisition only). The sample temperatures were maintained at approximately 30 °C during the measurements. The chemical shifts are reported in ppm upfield of external 1 M $D^{15}NO_3$ and are accurate to ±0.1 ppm. The coupling constants are accurate to ±1 Hz.

cis-$[Pt(NH_3)_2Cl_2]$. ^{15}N-Labeled *cis*-$[Pt(NH_3)_2Cl_2]$ was prepared as described previously (Lebedinskii & Golovnya, 1947) using 99% ^{15}N-enriched ammonium acetate (Stohler Isotope Chemicals) and potassium tetrachloroplatinate(II) (Alfa). Unlabeled *cis*-$[Pt(NH_3)_2Cl_2]$ was prepared by the method of Dhara (Dhara, 1970).

$K[Pt(NH_3)Cl_3]$. ^{15}N-Labeled $K[Pt(NH_3)Cl_3]$ was prepared from 99% ^{15}N-enriched ammonium acetate and potassium tetrachloroplatinate(II). To 900 mg of potassium tetrachloroplatinate(II) dissolved in 5 mL of water was added 250 mg of 99% ^{15}N-enriched ammonium acetate. The solution was boiled for 1.5 h and then cooled to room temperature, and some insoluble *cis*-$[Pt(NH_3)_2Cl_2]$ was removed by filtration. About 5 mL of ethanol was added to precipitate the excess ammonium acetate. The ^{15}N-labeled $K[Pt(NH_3)Cl_3]$ was not isolated but was characterized by means of its ^{15}N spectrum.

$[Pt(NH_3)_3Cl]^+$. ^{15}N-Labeled $[Pt(NH_3)_3Cl]^+$ was prepared from ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$. To 50 mg of 99% ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ dissolved in 20 mL of water was added 28 mg of silver nitrate. The solution was heated to about 60 °C for 10 min and then filtered to remove the precipitated silver chloride. Formation of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ was confirmed by the ^{15}N spectrum of the solution. To this solution was added 15 mg of 99% ^{15}N-labeled ammonium acetate, and the mixture was boiled for 1 h. The formation of ^{15}N-labeled $[Pt(NH_3)_3Cl]^+$ was confirmed by the ^{15}N spectrum of the solution. No attempt was made to isolate the product.

Monopyridine Complexes. A mixture of the ^{15}N-labeled chloroamine complex and an equimolar amount of pyridine in water was allowed to stand at room temperature for approximately 1 day before taking the ^{15}N spectrum.

Aquo–Pyridine Complexes. To the solution of the monopyridine complex was added 2 equiv of silver nitrate. The mixture was heated to 50–60 °C for approximately 15 min and then filtered before taking the spectra.

Bis- and Tris(pyridine) Complexes. To the solution of either the monopyridine or aquopyridine complex was added an excess of pyridine. The solution was allowed to stand at room temperature for about 1 day before taking the spectra.

cis-$[Pt(NH_3)_2anCl]^+$. To 50 mg of 99% ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ in 20 mL of water was added an equimolar amount, 16 mg, of 95% ^{15}N-labeled aniline (Merck Sharp & Dohme, Inc.). The solution used for the spectra was heated to 70–80 °C for approximately 15 min.

cis-$[Pt(NH_3)_2(an)_2]^{2+}$. To the solution of ^{15}N-labeled *cis*-$[Pt(NH_3)_2anCl]^+$ was added 2 additional equiv (32 mg) of ^{15}N-labeled aniline. The solution was heated to 70–100 °C for approximately 30 min. The ^{15}N spectrum of this solution showed that only 60% of the *cis*-$[Pt(NH_3)_2anCl]^+$ was converted to *cis*-$[Pt(NH_3)_2(an)_2]^{2+}$.

Monochloro–Mononucleoside Complexes. To 100 mg of 99% ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ in 25 mL of water was added 1 molar equiv of the nucleoside. The solution used for the ^{15}N spectra was heated to dissolve the nucleoside and then allowed to stand at room temperature or heated to 70–80 °C for 30 min.

Monoaquo–Mononucleoside Complexes. To the solutions of the monochloro–mononucleoside complexes was added about 2.2 molar equiv of silver nitrate. The solutions were heated to 50–60 °C for about 15 min and then filtered to remove the solid silver chloride. With a few of the samples, some colloidal silver chloride remained.

Bis(nucleoside) Complexes. To solutions of either the monochloro–mononucleoside complex or the monoaquo–mononucleoside complex was added a second molar equivalent of the nucleoside. The mixtures were allowed to stand at room temperature for 1 day or heated to 70–80 °C for 30 min before taking the ^{15}N spectra.

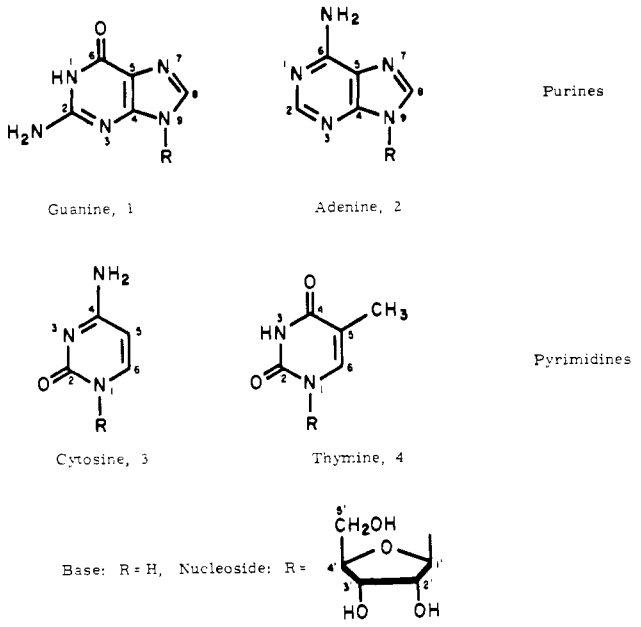
Unlabeled Bis(nucleoside) Complexes. Mixtures of 1.9 g of *cis*-$[Pt(NH_3)_2Cl_2]$ and 2 molar equiv of nucleoside in 500 mL of water were heated to 50–60 °C for approximately 12 h. The solutions used for taking the spectra were concentrated to about 25 mL on a rotary evaporator.

Results and Discussion

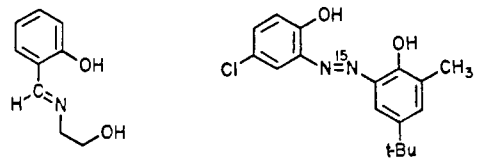
Model Complexes. The DNA bases include two purines, guanine (**1**) and adenine (**2**), and two pyrimidines, cytosine (**3**) and thymine (**4**). Because each base has several potential platinum-binding sites, more than one complex could be formed from *cis*-$[Pt(NH_3)_2Cl_2]$ and any one of the nucleosides.

The spectra were taken of several model platinum complexes, to aid in the interpretation of the ^{15}N spectra of the platinum–nucleoside complexes. Pyridine was chosen as a model for the azine-type unsaturated nitrogens of the nucleosides (e.g., N1 of adenosine) and aniline was used as a model for the exocyclic amine sites (e.g., the $-NH_2$ at C2 of guanosine). The ammonia ^{15}N chemical shifts and coupling constants of the model platinum complexes are listed in Table I.

^{15}N Chemical Shifts. The ^{15}N chemical shifts of ammonias complexed to platinum can be seen from Table I to depend on the nature of both the trans and cis ligands. Some cis and trans substituents effects on the ammonia chemical shifts calculated from the data in Table I are listed in Table II. The trans ligands clearly have large influences on the ammonia chemical shifts, and the relative order of the trans ligand



induced downfield shifts is $NH_3 > Cl^- \sim C_6H_5NH_2 \sim C_5H_5N > H_2O$. Pregosin and co-workers (Motschi & Pregosin, 1980; Motschi et al., 1979) have observed similar trends with platinum complexes of 1-hexanamine and Schiff base **5**. It



is further apparent that the less weakly bound trans ligands tend to cause the nitrogen resonances to move upfield. Cis ligands generally have lesser and opposite influences on the position of the ammonia nitrogen resonances. The relative order of the cis ligand induced downfield shifts is almost the reverse of the trans-ligand order.

The ^{15}N chemical shifts of ammonia molecules complexed to platinum fall between 40 and 70 ppm *upfield* of the ^{15}N resonance of free ammonia. This is opposite in direction to the usual protonation shifts of primary amines (Roberts, 1980). Large upfield platinum coordination shifts are also observed for the nitrogen resonances of pyridine–platinum and aniline–platinum complexes (see Table III), and these are more in accord with protonation shifts. Platinum coordination shifts have also been reported for complexes with 1,2-diaminoethane (Alei et al., 1979), 1-methylimidazole (Alei et al., 1979), the single ^{15}N-labeled diarylazo ligand **6** (Pregosin and Steiner, 1976), the Schiff base (**5**; Motschi & Pregosin, 1980), and 1-hexanamine (Motschi et al., 1979). A linear relationship is evident (Figure 1) between the platinum coordination shifts and the chemical shifts of the free ligand for a constant trans ligand.

One approach to analyzing nuclear magnetic resonance shieldings is to regard them as being the sum of three terms (Levy & Lichter, 1979; Saika & Slichter, 1954; Randall & Gillies, 1971):

$$\sigma^A = \sigma_d{}^A + \sigma_p{}^A + \sum_{B \neq A} \sigma^{AB} \quad (1)$$

Here, σ^A is the shielding constant of the nucleus concerned, $\sigma_d{}^A$ is the local diamagnetic term, $\sigma_p{}^A$ is the local paramagnetic term, and the $\sum_{B \neq A} \sigma^{AB}$ term (which is normally small for first-row elements and usually neglected) takes into account

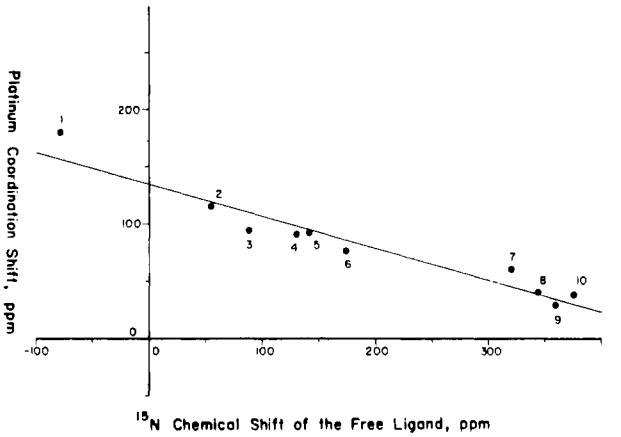


FIGURE 1: Plot of ^{15}N platinum coordination shifts vs. chemical shifts of free ligand. These shifts correspond to platinum complexes with a nitrogen ligand, either pyridine or ammonia, trans to the measured ligand. The least-squares fit gives a slope of −0.28, an intercept of 132 ppm, and a correlation coefficient, r, of 0.957. The individual points are taken from the present work and papers cited in the text. (1) **6**; (2) pyridine; (3) **5**; (4) 1-methylimidazole; (5) guanosine, N7; (6) cytidine, N3; (7) aniline; (8) 1-hexanamine; (9) 1,2-diaminoethane; (10) ammonia.

the shielding effects of other atoms in the molecule and medium effects.

The diamagnetic contribution to the chemical shielding has been approximated (in SI units) by

$$\sigma_d{}^k = \sigma_d(\text{free atom/free ion}) + (e^2/30\ m)\sum_\alpha{}'(Z_\alpha/r_\alpha) \quad (2)$$

where $\sigma_d{}^k$ is the diamagnetic contribution to the shielding of the kth nucleus in a molecule, σ_d(free atom/free ion) is the diamagnetic shielding of the free atom or ion (depending upon whether the atom in question bears a formal charge), Z_α is the atomic number of the αth nucleus, r_α is the distance between the nuclei k and α, and the summation runs over all atoms directly bound to nucleus k (Grinter & Mason, 1970; Flygare & Goodisman, 1968). The equation has been used to calculate the diamagnetic contribution to the nitrogen shieldings in small organic molecules (Grinter & Mason, 1970; Christl et al., 1973; Warren & Roberts, 1974).

The paramagnetic contribution is usually expressed in the form (Levy & Lichter, 1979)

$$\sigma_p{}^A \propto \Delta E^{-1}\langle r^{-3}\rangle_{2p}\sum_B Q_{AB} \quad (3)$$

where ΔE is the average excitation energy, $\langle r^{-3}\rangle_{2p}$ is the mean value of the inverse cube of the 2p orbital radius, and the Q_{AB} terms (where B is the neighbor of A) contain the elements of the charge-density/bond-order matrix. These terms are not easy to evaluate; however, qualitative arguments about the magnitude of the paramagnetic contribution can be made from eq 3.

The cancelling of a large diamagnetic shielding by a nearly equal, large opposing paramagnetic deshielding has been invoked (Hagen et al., 1973) to account for the small ^{15}N changes occuring on complexation of metals to EDTA. However, it has been suggested (Bose & Abbot, 1977) that the paramagnetic deshielding terms are probably not large because the ^{15}N chemical shieldings in a series of rhodium(III) complexes do not correlate with the position of the ligands in the spectrochemical series. Nitrogen shift changes on platination have been hypothesized to result from second-order paramagnetic effects, similar to those proposed for the ^{15}N protonation shifts (Motschi & Pregosin, 1980). However, it seems more likely that such shifts are the result of several

Table I: ^{15}N Chemical Shifts and Coupling Constants of Coordinated Ammonias

compound	$\delta_{^{15}N}$ (ppm) trans Cl	trans N	trans H_2O	$^1J_{^{15}N-^{195}Pt}$ (Hz) trans Cl	trans N	trans H_2O
cis-$[Pt(NH_3)_2Cl_2]$	422.1			325		
cis-$[Pt(NH_3)_2(H_2O)Cl]^+$	420.2		443.2	343		368
cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$			440.2			388
$[Pt(NH_3)Cl_3]^-$	420.4			324		
$[Pt(NH_3)(H_2O)_3]^{2+}$			442.4			387
$[Pt(NH_3)_3Cl]^+$	424.2	420.4		329	282	
$[Pt(NH_3)_4]^{2+}$		421.2			286	
cis-$[Pt(NH_3)_2pyCl]^+$	417.6	423.5		343	273	
cis-$[Pt(NH_3)_2py(H_2O)]^{2+}$		421.3	436.8		290	384
cis-$[Pt(NH_3)_2(py)_2]^{2+}$		419.6			288	
$[Pt(NH_3)(py)_3]^{2+}$		414.7			302	
$[Pt(NH_3)_3py]^{2+}$		416.1 (NH_3), 424.7 (py)			295 (NH_3), 277 (py)	
cis-$[Pt(NH_3)_2anCl]^+$	418.4	423.1		351	287	
cis-$[Pt(NH_3)_2(an)_2]^{2+}$		418.4			307	

Table II: Range of Ligand Effects on ^{15}N Chemical Shifts

ligand	ppm[a] trans	cis
H_2O	+19.2 to +21.1 (+20.1)	−3.0 to −0.9 (−2.0)
pyridine	+0.5 to +2.0 (+1.2)	−6.4 to −3.9 (−4.8)
aniline	0.0 to +1.0 (+0.5)	−4.7 to −3.7 (−4.2)
Cl^-	0	0
NH_3	−3.0 to −0.7 (−1.7)	+0.8 to +2.1 (+1.4)

[a] Average values in parentheses.

Table III: ^{15}N Chemical Shifts of Coordinated Ligands and Free Ligands

compound	$\delta_{^{15}N}$ (ppm)
pyridine (neat)	56.7
cis-$[Pt(NH_3)_2(py)_2]^{2+}$	174.6
aniline (aqueous)	319.0
cis-$[Pt(NH_3)_2anCl]^+$	378.2
cis-$[Pt(NH_3)_2(an)_2]^{2+}$	380.6

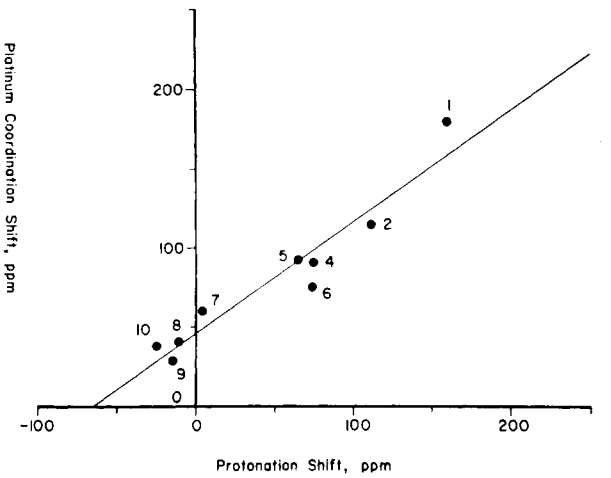


FIGURE 2: Plot of ^{15}N platinum coordination shifts vs. ^{15}N protonation shifts of free ligand. A least-squares fit yields a slope of 0.71, an intercept of 46 ppm, and a correlation coefficient, r, of 0.961. The points are numbered to correspond in the same way as in Figure 1, except the protonation shift of azobenzene was used for point 1.

effects (Mason, 1981). The reason is that, while the ^{15}N platinum-coordination shifts of azine-type nitrogens appear to be linearly related to the corresponding ^{15}N protonation shifts (Figure 2), two effects seem to be involved. One of these is a diamagnetic shielding of approximately 45 ppm, which is the intercept of the correlation line of Figure 2. The other is a paramagnetic deshielding that becomes smaller on platinum coordination and amounts to about 70% of the corresponding change on protonation.

The trans influence of the ligands, as mentioned earlier, is large on the ^{15}N chemical shifts of other nitrogens complexed to platinum. Those trans ligands that bind strongly to platinum act to weaken the platinum–nitrogen bond and thus result in longer platinum–nitrogen bond distances (Langford & Gray, 1965; Belluco, 1974). Because diamagnetic shieldings are inversely related to the bond distances between nitrogen and neighboring nuclei, the diamagnetic shielding of a given nitrogen ligand is expected to decrease as the binding power of the ligand trans to it increases. The observed shieldings do indeed increase with the more loosely bound trans ligands, which is the expected trend. The binding of the trans ligands also affects the s character of the platinum–nitrogen bond (Pregosin et al., 1973), and hence, the nitrogen 2p orbital radius increases with the less-binding ligands. As a result, the paramagnetic deshielding of a nitrogen bound to platinum should decrease with the more weakly bound trans ligands, because the paramagnetic deshielding is inversely proportional to the cube of the nitrogen 2p orbital radius.

^{15}N–^{195}Pt Coupling Constants. The sizes of the one-bond ^{15}N–^{195}Pt coupling constants listed in Table I, like the ^{15}N chemical shifts, are dependent on the nature of the trans and cis ligands. In general, nitrogen ligands with ligands with smaller trans influences on the shift are associated with larger values of $^1J_{^{15}N-^{195}Pt}$. The one-bond ^{15}N–^{195}Pt coupling constants are directly related to the s character of the nitrogen and platinum orbitals (Motschi et al., 1979; Motschi & Pregosin, 1980; Al-Najjar et al., 1979). The observed coupling constants fall into three ranges: 270–310 Hz for those nitrogens bound to platinum that are trans to nitrogen ligands, 320–355 Hz for nitrogens trans to chloride, and 365–390 Hz for nitrogens trans to water. The effects of ligands on one-bond ^{15}N–^{195}Pt coupling constants of *trans*- and *cis*-ammonias derived from Table I are listed in Table IV. In addition to a large trans-ligand substituent effect, a significant cis-ligand substituent effect can be seen on the platinum–ammonia coupling constants. However, too few complexes were measured to determine whether a valid quantitative relationship exists between the trans-ligand and cis-ligand substituent effects and the nature of the ligands.

Nucleoside Complexes. Cytidine with an equimolar amount of ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ forms a monocytidine com-

Table IV: Range of Ligand Effects on $^1J_{^{15}N-^{195}Pt}$ Couplings in Hertz

ligand	trans [a]	cis [a]
H_2O	+41 to +45 (+43)	+17 to +20 (+43)
Cl^-	0	0
aniline	−44 to −38 (−41)	+21 to +26 (+24)
NH_3	−48 to −43 (−44)	+1 to +4 (+3)
pyridine	−55 to −52 (−53)	+13 to +18 (+16)

[a] Average values in parentheses.

Table V: ^{15}N Chemical Shifts and Coupling Constants of Ligands in Platinum-Nucleoside Complexes

compound	trans ligand	$\delta^{15}N$ [a] (ppm)
cis-$[Pt(NH_3)_2CytCl]^+$	Cl^-	420.7 (339)
	Cyt, N3	423.9 (300)
cis-$[Pt(NH_3)_2(Cyt)_2]^{2+}$	Cyt, N3	421.5 (304)
cis-$[Pt(NH_3)_2GuoCl]^+$	Cl^-	420.1 (338)
	Guo, N7	422.8 (298)
cis-$[Pt(NH_3)_2(Guo)_2]^{2+}$	Guo, N7	420.8 (310)
cis-$[Pt(NH_3)_2Guo(H_2O)]^{2+}$	Guo, N7	420.9 (321)
	H_2O	439.3 (376)
cis-$[Pt(NH_3)_2GuoCl]^+$	Cl^-	419.7
	Guo, N?	424.1
cis-$[Pt(NH_3)_2Guo(H_2O)]^{2+}$	Guo, N?	418.0
	H_2O	438.5
cis-$[Pt(NH_3)_2(Guo)_2]^{2+}$	Guo, N?	423.9

[a] $^1J_{^{15}N-^{195}Pt}$ values in hertz are given in parentheses.

plex, *cis*-$[Pt(NH_3)_2CytCl]^+$. The ^{15}N spectrum of this complex shows two resonances at 420.7 and 423.9 ppm, with couplings to ^{195}Pt of 339 and 300 Hz, respectively. The ^{15}N chemical shifts and coupling constants of the platinum–cytidine complexes are listed in Table V. From the values of the coupling constants, the resonance with shift 420.7 ppm can be assigned to the ammonia trans to the chloride ligand and the resonance with shift 423.9 ppm to the ammonia trans to cytidine (bound through a nitrogen). Cytidine reacts relatively slowly with *cis*-$[Pt(NH_3)_2Cl_2]$ and, even after 9 days, some starting material is detectable in the reaction mixture.

The monocytidine complex reacts with a second equivalent of cytidine to give a new resonance at 421.5 ppm. That only one resonance appears, when 2 equiv of cytidine was added to the ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$, indicates the formation of a symmetric bis(cytidine) complex where both cytidines are coordinated to platinum at the same cytidine nitrogen. The bis(cytidine) complex also forms very slowly and, even after a week, monocytidine complex still predominates. The relatively slow reaction of cytidine with *cis*-$[Pt(NH_3)_2Cl_2]$ agrees with earlier reports (Horacek & Drobnik, 1971; Robins, 1973).

The pattern of ^{15}N coupling constants of the monocytidine complex indicates that cytidine acts only as a monodentate ligand through a nitrogen atom. If chelate binding were to occur, involving the NH_2 at C4 and N3, then both coupling constants would be between 270 and 310 Hz (Al-Najjar et al., 1979). Further, if chelation involves N3 and the oxygen at C2, then the coupling constant of the ammonia trans to the carbonyl would be greater than 350 Hz (Al-Najjar et al., 1979; Kerrison & Sadler, 1981). Instead, the monocytidine complex clearly retains the chloride complexed to platinum, as evidenced by the ammonia ^{15}N to ^{195}Pt coupling of 339 Hz.

The ^{15}N spectra of the ammonia ligands alone do not allow one to decide whether N3 or the NH at C4 is involved in binding to platinum. The nitrogens of the coordinated cytidine can be observed directly by ^{15}N NMR at the natural-abundance level, and the chemical shifts of the bis(cytidine) complex are listed in Table VI. Comparison of these shifts with those of free cytidine 5′-phosphate (Markowski et al., 1977) shows that the resonance of N1 is unchanged, while that of N3 shifts 76 ppm upfield and that of the NH_2 11 ppm downfield relative to that of the free base. The large upfield shift of N3 upon coordination indicated that this is the nitrogen that binds to platinum. The shifts occurring on coordination are of the same magnitude and direction as those that take place on protonation shifts (Markowski et al., 1977; Büchner et al., 1978).

Table VI: ^{15}N Chemical Shifts of Nucleoside Nitrogens Alone and in Platinum-Nucleoside Complexes

compound	$\delta^{15}N$ (ppm) N1	N3	N7	N9	NH_2
cytidine 5′-phosphate [a]	223.5	175.2			283.2
cis-$[Pt(NH_3)_2(Cyt)_2]^{2+}$	223.6	251.0			272.4
guanosine 5′-phosphate [a]	229.1	211.3	141.2	207.2	303.5
cis-$[Pt(NH_3)_2(Guo)_2]^{2+}$	228.1	211.4	233.5	202.7	300.3

[a] Data from Markowski et al. (1977).

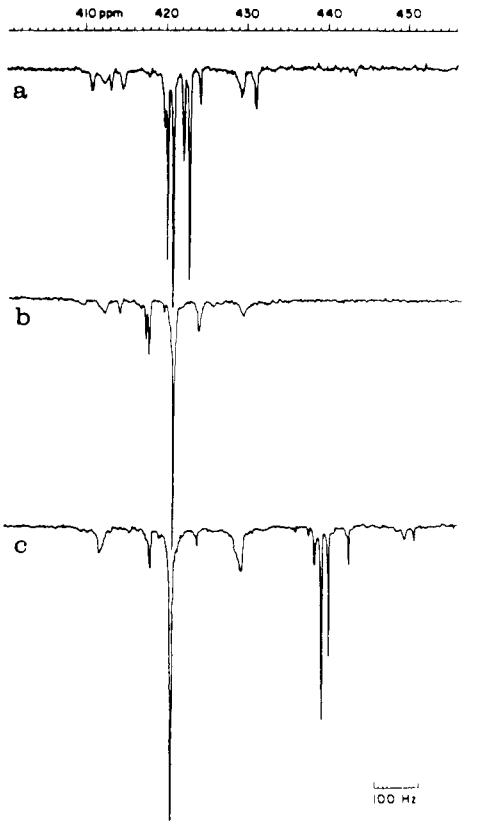
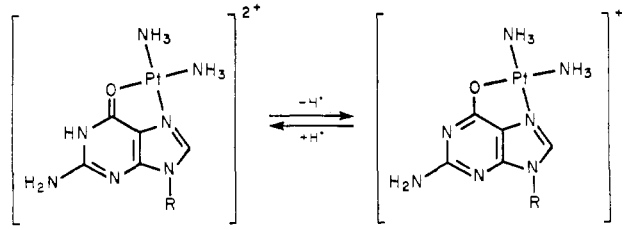


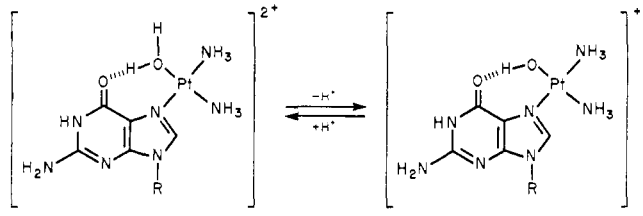
FIGURE 3: (a) ^{15}N spectrum of 100 mg (0.33 mmol) of ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ and 94 mg (0.33 mmol) of guanosine in 20 mL of water after heating at 50–60 °C for 1 hr. Acquisition parameters were 8200 scans, a 45° pulse width, and a 2-s repetition rate. (b) ^{15}N spectrum of a similar sample as (a) after an additional 94 mg of guanosine was added and the sample was left standing at room temperature for 3 days. Acquisition parameters were 20 000 scans, a 77° pulse width, and a 3-sec repetition rate. (c) ^{15}N spectrum of the same sample as (a) except after treatment with 113 mg (0.67 mmol) of silver nitrate. Acquisition parameters were 22 750 scans, at 45° pulse width, and a 2-s repetition rate.

When guanosine reacts with ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$, quite complex ^{15}N spectra are obtained, which indicate the formation of a mixture of guanosine complexes (see Figure 3). The spectrum from guanosine and an equimolar amount of ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ is shown in Figure 3a. Six resonances at 419.7, 420.1, 420.8, 422.1, 422.8, and 424.1 ppm,

Scheme I



Scheme II



respectively, were observed. The resonance at 422.1 ppm can be assigned to unreacted *cis*-$[Pt(NH_3)_2Cl_2]$. This 1:1 mixture, when treated with a second equivalent of guanosine, gave the spectrum shown in Figure 3b. Another 1:1 sample was treated with 2 equiv of silver nitrate to replace chlorides coordinated to platinum by water. The spectrum of the resultant products is shown in Figure 3c.

Comparison of the chemical shifts, coupling constants, and peak areas of the resonances of the 1:1 mixture with those of the 2:1 and 1:1 aquo mixture allowed assignment of most of the resonances to different guanosine complexes. The ^{15}N chemical shifts and coupling constants (when observable) of the platinum–guanosine complexes are listed in Table V. Clearly, the spectrum of the 1:1 aquo mixture (Figure 3c) allows inference that more than one monoguanosine–monochloro complex is formed. There are four resonances in the upfield region of the spectrum. The resonance at 440.2 ppm can be assigned to *cis*-$[Pt(NH_3)_2(H_2O_2)]^{2+}$, while the remaining resonances at 438.5, 439.3, and 442.7 ppm arise from monoguanosine–monoaquo complexes. The spectrum of the 2:1 mixture shows that a symmetric bis(guanosine) complex (with a shift of 420.8 ppm) is the major product formed. Other bis(guanosine) complexes, both symmetric and unsymmetric, are also formed, as indicated by the smaller resonances around the major resonance of the bis(guanosine) complex.

As with cytidine, there is no evidence that guanosine acts as a bidentate ligand in its reactions with *cis*-$[Pt(NH_3)_2Cl_2]$, through either two nitrogen sites (the NH_2 at C2 and N1 or N3) or the controversial binding through N7 and the oxygen at C6 (Goodgame et al., 1975; Millard et al., 1975). Despite this, the chemical shifts of the resonances assigned to *cis*-$[Pt(NH_3)_2Guo(H_2O)]^{2+}$ might be regarded as consistent with a chelate complex involving N7 and the oxygen at C6 (see Scheme I). The rationale here is that while the carbonyl oxygen is not likely to be a reactive enough ligand displace a coordinated chloride, it might well be able to displace a more weakly bound water. Thus, on removal of the chloride ligand with silver nitrate, the chelate complex could form in place of the monoguanosine–monoaquo complex.

The resonance of the ammonia trans to the oxygen ligand was found to be pH dependent, and this indicates that the oxygen ligand has acidic protons. The acidic proton could be either one at a coordinated water (see Scheme II) or the amide proton at N1 of coordinated guanosine (Scheme I).

However, the pK_a value of 4.9 for the acidic group (calculated by an iterative procedure that determines the limiting shifts and the pK_a best fitting the observed data) is as expected for a platinum-coordinated water (Gel'fman et al., 1974). The pK_a of the amide proton in free guanosine is about 9.2, and it seems unlikely that platinum coordination of the carbonyl oxygen at C6 would increase its acidity by more than 4 pK_a units.

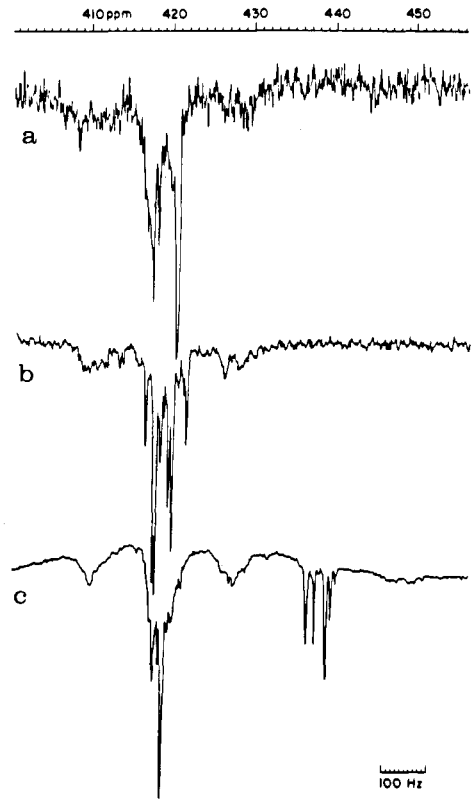


FIGURE 4: (a) ^{15}N spectrum of 100 mg (0.33 mmol) of ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ and 89 mg (0.33 mmol) of adenosine in 25 mL of water after standing at room temperature for 9 days. Acquisition parameters were 1635 scans, a 77° pulse width, and a 3-s repetition rate. (b) ^{15}N spectrum of the same sample as (a) after an additional 89 mg of adenosine was added and the sample was left standing at room temperature for 7 days. Acquisition parameters were 7820 scans, a 45° pulse width, and a 3-s repetition rate. (c) ^{15}N spectrum of a similar sample as (a) except after treatment with 150 mg (0.88 mmol) of silver nitrate. Acquisition parameters were 11 740 scans, at 60° pulse width, and a 5-s repetition rate.

The ^{15}N chemical shift of the base nitrogens of *cis*-$[Pt(NH_3)_2(Guo)_2]^{2+}$ are listed in Table VI. Comparison of these shifts with those of guanosine 5′-phosphate (Markowski et al., 1977) shows that the guanosines are bound to platinum through N7, because the N7 resonance shifts 92 ppm upfield, while the other resonances either remain unchanged or shift slightly downfield. The behavior of the resonances upon platination is again similar to changes observed on protonation (Markowski et al., 1977; Büchner et al., 1978).

It is interesting that substantial amounts of the symmetric bis(guanosine) complex are formed even in the 1:1 mixture of guanosine and *cis*-$[Pt(NH_3)_2Cl_2]$. Unlike cytidine, guanosine readily forms a symmetric bis(nucleoside) complex with *cis*-$[Pt(NH_3)_2Cl_2]$. Both guanosine and pyridine form bis(base) complexes in competition with the monobase complexes in the 1:1 mixtures. It is possible that base stacking interactions in solution makes the formation of these bis(base) complexes more favorable.

Adenosine and ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ yield what are clearly several complexes (see Figure 4). Although the spectrum of the 1:1 mixture shown in Figure 4a seems relatively simple, it is actually a composite of resonances from several monoadenosine complexes. Addition of a second

equivalent of adenosine to the 1:1 mixture yields a complicated mixture of bis(adenosine) complexes, as can be seen from Figure 4b. Treatment of the 1:1 mixture with silver nitrate yields the monoadenosine–monoaquo complexes shown in Figure 4c. It is clear from the four upfield resonances of the 1:1 aquo mixture that all four possible monoadenosine complexes were formed, but the spectra are too complicated to allow assignment of individual resonances. Extrapolation of the results from the 1:1 aquo mixture suggests most, if not all, of the possible bis(adenosine) complexes are formed in the 2:1 mixture. This certainly accords with the complexity of the 2:1 mixture, which is simply not well enough resolved to allow useful assignments.

Thymidine and uridine both react very slowly with *cis*-$[Pt(NH_3)_2Cl_2]$ to eventually form "platinum blues" (Davidson et al., 1975). These complexes are believed to be oligomers with platinum–platinum bonds (Lippard, 1978). The ^{15}N NMR shifts of the platinum-bound ammonias in solutions where either thymidine or uridine was allowed to react at room temperature for 2 weeks with ^{15}N-labeled *cis*-$[Pt(NH_3)_2Cl_2]$ showed that most of the *cis*-$[Pt(NH_3)_2Cl_2]$ remained unreacted.

Conclusions. ^{15}N NMR is clearly a powerful tool for determining the binding interactions of *cis*-$[Pt(NH_3)_2Cl_2]$ with nucleotides. Although the ^{15}N spectra of the base nitrogens are more informative about the details of the binding, the easily prepared ^{15}N-labeled drug allows spectra to be taken of a minimum amount of material. Certainly, study of the ammonia nitrogen resonances does allow determination of the number and type of ligands bound to platinum, and it is possible that the technique could be useful for the binding of *cis*-$[Pt(NH_3)_2Cl_2]$ to DNA itself.

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